REMARKS

The Applicants respectfully request reconsideration of claims 1 – 9, 11-17, 36, and 48 – 56, in light of the amendments and arguments submitted herewith.

Amendments to Claims 1 and 11

Claim 1 is amended to add, in the "introducing" step the phrase "*a transfection preparation comprising*" and is amended to add the limitation "*wherein (a)* the polynucleotide *is* operably linked to a promoter and *contains* a gene expression altering sequence ... *and (b) the transfection preparation further comprises one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, a cationic lipid agent.*" Support for this limitation is found in the application on p. 3, lines 11-14 ("introducing polynucleotides into cells may be facilitated by formulations that include a cationic lipid reagent, cationic non-lipid polymer transfection reagent ...") and p. 12, lines 1-6 ("... include cationic lipid reagents, non-liposomal formulations, and cationic polymer transfection reagents ....") and original claims 7-9.

Claim 11 is amended in the "introducing" step to read "introducing into the population of cells *a transfection preparation comprising* a DNA sequence operably linked to a promoter..." and amended to add the limitation "*wherein the transfection preparation further comprises one or more transfection reagents selected from the group consisting of cationic polymer agents*" after the introducing step. Support for this limitation is found in the application on p. 3, lines 16-17 ("... introducing into the population of cells ... in the presence of a cationic polymer ...") and p. 11, lines 31-32 ("... we found that ... improved results were obtained by transfection in the presence of cationic polymers.") and original claims 7, 11, 30, 37 and 39.

Cancelled Claims and Amendment to Claim 56

Claims 10, 22, and 23 are cancelled without prejudice, the Applicants reserving the right to prosecute such claims in a follow-on application. Claim 56 is amended to

correct a typographical error, replacing the word "method" with the phrase "reagent cell population" as is evident from the context of the claim.

Indefiniteness/Dependence from Claim 1

Claim 48 is amended to delete the term "foreign" from the claim. As such, the claim specifically delineates what is meant by "genetic material," and complies with the requirements of 35 U.S.C. §112, second paragraph.

In addition, claims 36 and 48 are amended to depend from claim 1, by adding the phrase "having altered gene expression produced in accordance with claim 1" in the preamble of the claim.

No New Matter

Applicants respectfully submit that no new matter has been added with any of the amendments to the claims detailed above.

Prior Art Rejections

1. Anticipation

Smith teaches the use of electroporation to transfect murine embryonic stem cells. Smith does not teach a method of altering gene expression in a population of human embryonic stem (ES) cells "wherein ... the transfection preparation further comprises one or more agents selected from the group consisting of a cationic non-lipid polymer agent, a non-liposomal agent, a cationic lipid agent" as required by claim 1, as herein amended. Thus Smith cannot anticipate claim 1 or claims 2-4, 6, 36, and 48-56 which depend from claim 1.

For similar reasons, Smith does not anticipate claim 11, because Smith does not teach a method or altering gene expression in a population of human the embryonic stem (ES) cells "wherein the transfection preparation further comprises one or more

transfection reagents selected from the group consisting of cationic polymer agents" as required by claim 11, as herein amended.

2. Nonobviousness

To establish a *prima facie* case of obviousness three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available in the art, to modify the reference or to combine references. Second, there must be reasonable expectation of success, and third, the prior art reference (or combined references) must teach or suggest all the claim limitations. In the present case, the *prima facie* case fails because none of the three criteria are met. There is no suggestion in the references themselves or knowledge generally available to modify or combine any of the references, there is no reasonable expectation of success, and the prior art references, in any combination, do not teach or suggest all the claim limitations.

First, nothing in any reference suggests modifying the references, whether individually or together, to arrive at the claimed subject matter, nothing suggest combining them with other references to arrive at the claimed subject matter, and nothing in the general knowledge suggests the modifications or combinations either. As herein amended, claims 1 and 11, and all remaining claims which depend therefrom, require (i) transfecting human embryonic stem cells, (ii) introducing a transfection preparation comprising a polynucleotide, or DNA, the transfection preparation further comprising one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, and a cationic lipid agent, as required by amended claim 1, or comprising one or more transfection reagents selected from the group consisting of cationic polymer agents, as required by amended claim 11.

To arrive at the presently claimed subject matter, there would have to be a suggestion or the motivation to modify every known reference or protocol relating to transfection in mammalian cells so that one could achieve successful transfection in human ES cells. Then, there would have to be a suggestion or motivation to combine this modification with one or more references disclosing the use of a transfection reagent to introduce a polynucleotide or DNA into a population of human ES cells, also modified,

this time to use *only* one or more transfection reagents that meet the criteria of claims 1 and 11 and no other. And on top of all these very specific combinations and specific modifications of every single known technique or prior art reference relating to transfection in general and in mammals in particular to achieve successful transfection specifically in human embryonic stem cells, one would still have to alter gene expression in the resulting transfected human embryonic stem cells "so that gene expression in the embryonic stem cells prior to introducing the polynucleotide is measurably difference from gene expression after introducing the polynucleotide, while retaining the pluripotent character of the cells." See claims 1 and 11, emphasis added. It is just not believable that someone would find the motivation in the knowledge generally available to combine all the necessary references (protocols) and concomitantly modify each of them to arrive at all the limitations of the presently claimed invention, since there is no suggestion in any of the cited prior art references to modify them or combine them with another. As stated in MPEP §2143.01, "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination" (as paraphrased from *In re Mills*, 916 F.2d 680 (Fed.Cir. 1990)).

For example, regarding the Bradley/Thomson combination cited by the Examiner as rendering claims 1-17, 36, and 48-56 obvious, although Bradley et al. state that their invention may be performed with human cells, their methodology uses electroporation to transfect, and nothing is said in Bradley et al, regarding changing the protocol to a different method of transfection, particularly to the method required in the presently claimed invention wherein cationic polymer transfection reagents are introduced with the polynucleotide.

Similarly, Fasbender deals with transfection of genes into COS-1 cells (monkey kidney cell line), NIH-3T3 cells (mouse fibroblast cell line) or 9L gliosarcoma cells (rat muscle tumor cell line), not with transfection into human embryonic stem cell lines. None of the cell lines used in Fasbender were stem cells, let along human embryonic stem cells. One skilled in the art is not going to simply mix and match transfection protocols from such disparate references – namely, Smith, dealing with electroporation transfection methods in animal embryonic stem cells, and Fasbender, dealing with

adenovirus-coupled transfection techniques in the presence of cationic molecules in non-human, non-stem cell systems.

Further, Fasbender would have to be modified to eliminate the adenovirus from the transfection methodology, but there is no suggestion or motivation to do so, particularly when Fasbender states in the abstract that "[n]onviral cationic vectors ... do not catalyze the subsequent steps in gene transfer" – i.e., in the absence of adenovirus successful transfection does not occur. The Smith/Fasbender (and therefore Smith/Fasbender/Pascolo) combination and the required modifications – particularly removing adenovirus from the transfection protocol directly against the teachings of Fasbender – are only plausible using impermissible hindsight, an advantage not available when citing/combining references for an obviousness rejection. See MPEP §2145, subsection X. A. Further, if "the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification" (as paraphrased from In re Gordon, 733 F.2d 900 (Fed.Cir. 1984)). Since Fasbender specifically states that "[n]onviral cationic vectors ... do not catalyze the subsequent steps in gene transfer" (Abstract), then there is no motivation or suggestion to combine Fasbender to make the proposed modification to arrive at a transfection methodology that does not use adenovirus in the transfection protocol.

Second, regarding the §103 rejections of claims 7 and 11-17 by Smith and Fasbender (and Pascolo, for claim 17 only), there is no *prima facie* case of obviousness because there is no reasonable expectation of success. As detailed above, Fasbender deals with transfection of genes into COS-1 cells (monkey kidney cell line), NIH-3T3 cells (mouse fibroblast cell line) or 9L gliosarcoma cells (rat muscle tumor cell line), not with transfection into human embryonic stem cell lines. None of the cell lines used in Fasbender were stem cells, let along human embryonic stem cells. Smith teaches transfection of animal stem cells using electroporation. Assuming one skilled in the art found the motivation to make these combinations, and the motivation to modify both references extensively to arrive at a transfection protocol using transfection reagents selected from the group consisting of cationic molecules, and not using electroporation or adenovirus as a co-reagent for introducing the genetic material, one skilled in the art

would have no expectation that the resulting protocol would succeed in transfecting genetic material into hES cells.

Fasbender specifically states that "[n]onviral cationic vectors ... do not catalyze the subsequent steps in gene transfer" (see Abstract). Modifying Fasbender to remove the adenovirus co-reagent teaches away from the presently claimed subject matter. According to MPEP §2141.02, the "prior art must be considered in its entirety, i.e. as a whole, including portions that would lead away from the claimed invention" (as paraphrased from *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed.Cir. 1983)). Moreover, in the highly unpredictable field of stem cell research, one cannot assume that a protocol that works in one animal will work in another. Specifically, there is no expectation, given the poor transferability of mouse experiments to human experiments, that transfection procedures optimized for transfection in mice (or monkeys), none of which are for transfecting stem cells of any kind, can be modified to successfully transfect genetic material into human embryonic stem cells and result in the transfected genetic material altering gene expression in the hES cells but still retaining the pluripotent character of the hES cells.

As of the filing date of this application, no one had shown that mouse protocols for ES cells, whether for transfecting genetic material or for performing other manipulations, could be successfully translated to *human* ES cells. It is a trivialization of the ingenuity and unexpected results of the presently claimed invention to assume that protocols relating to non ES cells of lesser mammalians can be readily translated into successful protocols for ES cells in humans, particularly when it is typically not possible to translate protocols from even closely related species such as monkeys to human systems with any degree of certainty or predictability.

Third, claims 1-9 and 11-17, and 36, 48, 52, 54, and 56 are not obvious in light of any combination of the cited art because *none* of the cited art combinations teach all the limitations of independent claims 1 and 11 – namely, a method of altering gene expression in a population of *human* embryonic stem (ES) cells comprising introducing a transfection preparation comprising a polynucleotide, or DNA, the transfection preparation further comprising one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, and a

cationic lipid agent, as required by amended claim 1, or comprising one or more transfection reagents selected from the group consisting of cationic polymer agents, as required by amended claim 11.

Regarding claims 1 and 11, and claims which depend therefrom, the combination of Thomson and Bradley does not teach or suggest all the limitations of claims 1 and 11, as herein amended. Thomson teaches the harvesting of human ES cells from the inner cell masses of blastocysts and Bradley et al. teach the transfection of *murine* embryonic stem cells using electroporation and homologous recombination. Neither reference, alone or in combination, teaches or suggests (i) transfecting *human* embryonic stem cells, as required by claims 1 and 11; and (ii) introducing a transfection preparation comprising a polynucleotide, or DNA, the transfection preparation further comprising one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, and a cationic lipid agent, as required by amended claim 1, or comprising one or more transfection reagents selected from the group consisting of cationic polymer agents, as required by amended claim 11.

In addition, claims 1 and 11, and dependent claims therefrom, are not obvious in light of Smith combined with Myers or combined with Fasbender, or combined with Fasbender and Pascalo. As previously discussed above regarding the anticipation rejection, Smith teaches the transfection of DNA into only *murine* embryonic stem cells using only electroporation, and Fasbender is drawn to using a cationic polymer or a cationic lipid in combination *with an adenovirus* to infect human epithelia or nasal epithelium of cystic fibrosis in *mice* (see abstract).

Myers and Pascolo are directed toward the teaching of specific fluorescent proteins and knockout genomic sequences, respectively, but do not provide any teaching about transfection of human embryonic stem cells. Thus, even when combined with Smith (Myers) or Smith and Fasbender (Pascolo) all of the claim limitations of the present invention are not taught or suggested by the combinations.

The combination of Smith with Gibco-BRL also does not teach all the claim limitations for the same reasons. Further, none of the combinations with Smith teach the claim limitations regarding a method of altering gene expression in a population of human embryonic stem cells comprising introducing a polynucleotide, or DNA, wherein

introducing the polynucleotide/DNA is facilitated by the presence of one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, and a cationic lipid agent, as required by amended claim 1, or by the presence of one or more transfection reagents selected from the group consisting of cationic polymer agents, as required by amended claim 11. Thus, none of the combinations with Smith can support a *prima facie* case of obviousness because all such combinations fail to teach or suggest all of the claim limitations of the presently claimed subject matter.

In summary, even if the combinations proposed by Examiner in the 103 obviousness rejections were made, one simply would not arrive at the subject matter claimed in the present application. As argued above, the prima *facie case* of obviousness fails for lack of motivation or suggestion to combine or modify the cited references to arrive at the presently claimed invention, fails for lack of a reasonable expectation of success, and especially fails because none of the combinations disclose all the limitations of the presently claimed invention. The Examiner has cited a multitude of combinations to attempt to establish a *prima facie* case of obviousness against the presently claimed invention, but the combinations do not hold up. Without the impermissible use of hindsight, one skilled in the art would not find the suggestion to combine and/or modify the teachings in the prior art of stem cell research – a hugely unpredictable field – to solve the problem of translating results in mice and monkeys to humans such that not only was it possible to efficiently transfect hES cells with genetic material using cationic polymer molecules, but the transfected hES cells also exhibited altered gene expression.

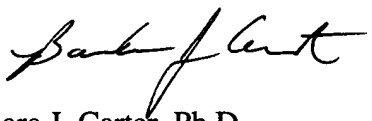
For all the foregoing reasons, Applicants respectfully submit that the pending claims are not obvious in light of the cited prior art. Reconsideration of the claims and withdrawal of the obviousness rejections are therefore requested

CONCLUSION

In view of the arguments and amendments presented, Applicants respectfully submit that all pending claims are now in condition for allowance. Reconsideration of the claims and a notice of allowance are therefore respectfully requested.

Applicants believe that a three-month extension of time is required and submit a petition for a three-month extension with this response, along with a Request for Continued Examination (RCE), and submit a check for $860 to cover the $475 owed for a three-month Extension Fee and the $385 fee owed for filing an RCE. In the event that any additional fees are required for the timely consideration of this application, please charge deposit account number 19-4972.

Respectfully submitted,



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